Introduction

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Skin

The skin is our most important organ; it protects and regulates the body in our complex environment. Its most important functions are to protect against external agents, regulate body temperature, harbor sensory nerves, and act as a capsule for the internal organs.[1] Besides being the largest organ of the human body, it is also very complex. The skin consists of the epidermis, the dermis, and the underlying connective and fatty tissue. The epidermis is the outermost layer of the skin that we can see, for example, while looking at our hands holding this thesis. It consists mainly of keratinocytes, which get their name from the protein keratin that is highly expressed throughout the epidermis.[2] Besides the keratinocytes, other cells are also found in the epidermis, whose main function is the protection against external agents. For example, Langerhans cells that provide protection by recognizing non-self molecules and melanocytes that secrete the pigment melanin.[3] Beneath the epidermis lies the dermis, which has a lower density of cells. The dermis provides flexibility and connectivity through a complex matrix of extracellular proteins.[4] This matrix comprises mainly the protein collagen and elastin, and is populated by scattered fibroblasts. The dermis also contains hair follicles, sweat glands, blood vessels, and nerves. These are all held in place by a matrix of connective tissue, which provides both toughness and flexibility, giving rise to the complex nature of the skin. Beneath the dermis lies more connective tissue and the adipose tissue, which is populated by cells that can store energy in the form of fatty lipids when the environment supplies less energy.[5]

A close look at the epidermis reveals that it can be divided into four layers, or strata: a compact dense layer of cells at the base (the stratum basale); a layer of faster proliferating cells that have a spiny appearance due to intercellular connections (stratum spinosum); a layer of fast differentiating cells of which the cytoplasm contains apparent granules (stratum granulosum); and the outer layer of cornified dead cells (stratum corneum). The junction between the epidermis and the dermis is called the basement membrane zone (BMZ), and this is the skin region that I will focus on in this thesis.

The basement membrane zone

The BMZ encompasses a complex network of proteins that together connect the epidermis to the dermis (Figure 1). There are many proteins intertwined in the BMZ and each of them connects to specific binding partners.[6-8] The BMZ has four major components: the basal keratinocytes, the lamina lucida, the lamina densa, and the sublamina densa.[6] The lamina lucida and lamina densa acquired their names because of their appearance under electron microscopy, i.e. their electron density.[9] The basal keratinocytes express proteins that assemble into so-called hemidesmosomes. These hemidesmosomes are transmembrane structures of the basal keratinocytes that connect them to the underlying lamina densa.

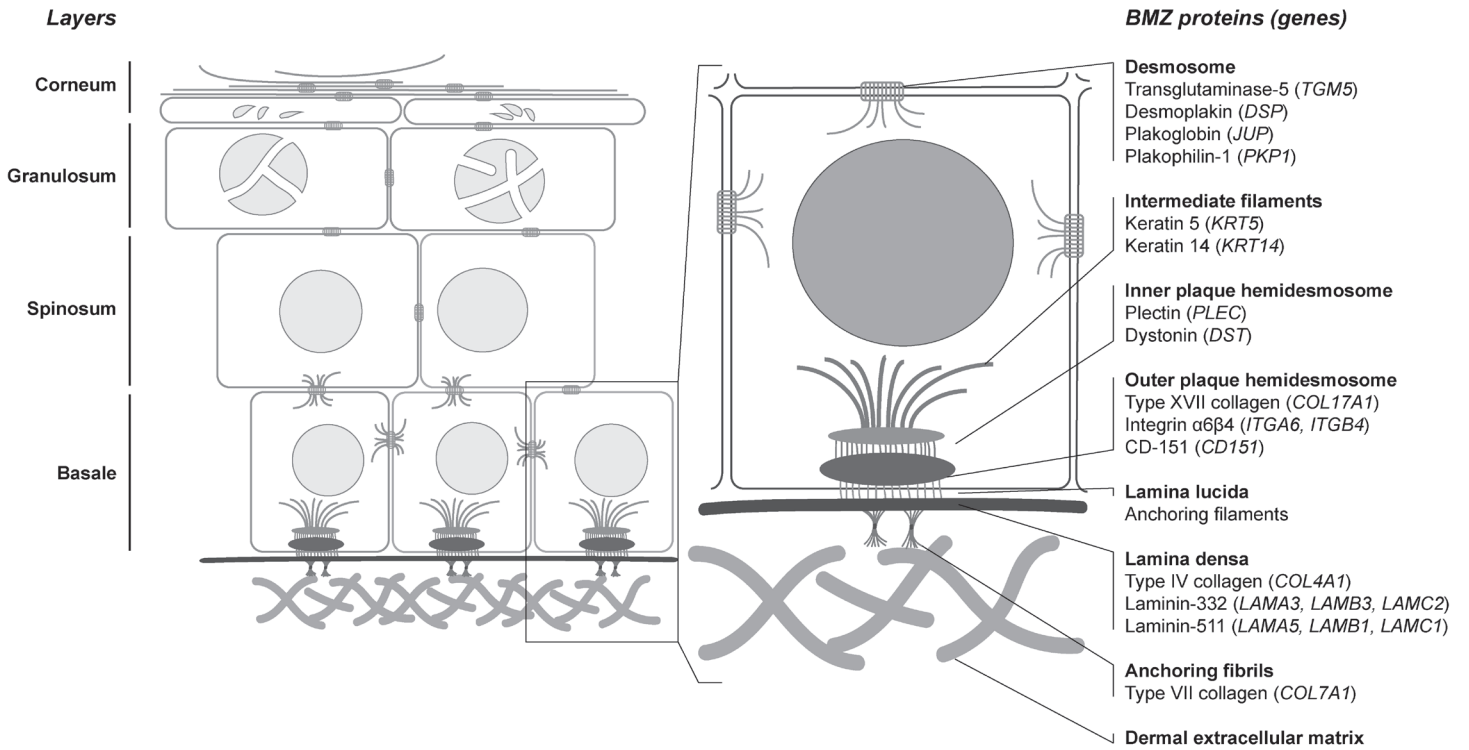

Figure 1. Schematic overview of the basement membrane zone. Left: The epidermal layers are shown. The stratum basale, spinosum, granulosum, and corneum are visualized. **Right:** magnification of a basal keratinocyte with a schematic representation of the location of the structural proteins involved in the basement membrane zone (BMZ). Desmosomes, intermediate filaments, hemidesmosomes, lamina lucida, lamina densa, anchoring fibrils and dermal extracellular matrix are indicated.

Each layer of the BMZ comprises multiple proteins that adhere to other proteins in different layers of the BMZ. Keratin 5 and keratin 14 are assembled into intermediate filaments that bind to plectin and dystonin in the cytoplasm of the basal keratinocyte.[10] Together they form the inner plaque of the hemidesmosome. The intracellular domain of the transmembrane-proteins – type XVII collagen, integrin $\alpha6\beta4$, and CD151 (tetraspanin 24) – make up the outer plaque of the hemidesmosome. The lamina lucida comprises the extracellular domains of type XVII collagen, integrin $\alpha6\beta4$, and CD151. The lamina densa consists mainly of type IV collagen and laminins, mainly laminin-332 and laminin-511. The extracellular domain of type XVII collagen and the integrin $\alpha6\beta4$ complex extend through the lamina lucida and bind to laminin-332 in the lamina densa. Type VII collagen binds mainly to laminin-332 and connects the lamina densa to the dermal matrix. In order to do this, type VII collagen aggregates laterally into so-called anchoring fibrils that connect the lamina densa to the uppermost part of the dermis (the papillary dermis). The main focus of this thesis is on type VII collagen and its coding gene *COL7A1*.

Epidermolysis bullosa

The genetic blistering disease epidermolysis bullosa (EB) is caused by pathogenic mutations in genes encoding proteins involved in the formation of the BMZ, or cell-cell adhesion in the epidermis.[11-13] The disease is characterized by blistering of the skin, and frequently of the mucosa, upon minor trauma. To date, there is consensus on the classification of 18 genes known to be involved in EB, although there are still unsolved cases and new genes are continually being discovered. It is most likely that at least a few new genes involved in EB will be discovered in the near future. Although it is not yet part of the consensus classification,[11] *CD151*, encoding tetraspanin-24, is associated with pretibial skin blistering and BMZ formation of epithelial tissue and kidney, and could be included as well.[12] Mutations in *KLHL24*, the kelch-like family member 24, have also been shown to cause EB simplex in several families,[14] while mutations in *FLG2*, encoding filaggrin-2, were recently shown to cause a generalized form of peeling skin syndrome, which can be regarded as a superficial form of EB, analogous to other peeling skin syndromes.[15] For the sake of completeness, and since mutations in these three genes also lead to blistering of the skin, we have include them in Table 1.

EB can be divided into four major subtypes: EB simplex (EBS), junctional EB (JEB), dystrophic EB (DEB), and Kindler syndrome. The subtypes are categorized by the level of blistering (Figure 2). In EBS, blistering occurs within the epidermis and a distinction is made in suprabasal and basal blistering. Suprabasal epidermal blistering is generally due to mutations in transglutaminase-5 (*TGM5*), desmoplakin (*DSP*), plakoglobin (*JUP*), or plakophilin 1 (*PKP1*). Basal epidermal blistering is usually due to mutations in keratin 5 (*KRT5*), keratin 14 (*KRT14*), exophilin-5 (*EXPH5*), CD-151 (*CD151*), plectin (*PLEC*), or dystonin (*DST*). Recently, the kelch-like 24 protein (*KLHL24*), has been reported to cause basal EBS

Table 1. List of 21 genes involved in EB and their usual level of blistering

Gene	Protein	Usual level of blistering
TGM5	Transglutaminase 5	Suprabasal epidermal
DSP	Desmoplakin	"
JUP	Plakoglobin	"
PKP1	Plakophilin 1	"
FLG2	Filaggrin-2	"
KLHL24	Kelch like family member 24	Basal epidermal
KRT5	Keratin 5	"
KRT14	Keratin 14	"
EXPH5	Exophilin 5	"
PLEC	Plectin	"
CD151	Tetraspanin-24	"
DST	Dystonin	"
LAMA3	Laminin alpha 3 chain	Intralamina lucida
LAMB3	Laminin beta 3 chain	"
LAMC2	Laminin gamma 2 chain	"
COL17A1	Type XVII collagen	"
ITGB4	Integrin beta 4	"
ITGA6	Integrin alpha 6	"
ITGA3	Integrin alpha 3	"
COL7A1	Type VII collagen	Sublamina densa
FERMT1	Kindlin-1	Mixed levels

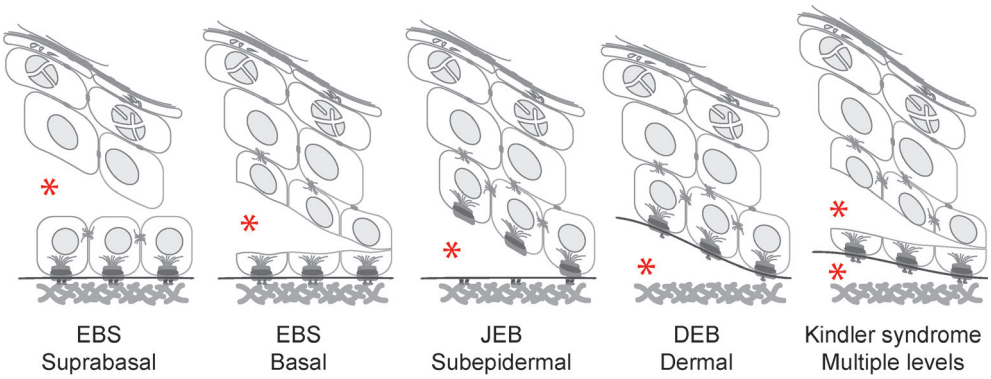

Figure 2. The levels of blistering in epidermolysis bullosa. The level of blistering in different subtypes of epidermolysis bullosa is schematically visualized by 'tearing upwards'. EBS epidermolysis bullosa simplex; JEB junctional epidermolysis bullosa; DEB dystrophic epidermolysis bullosa; Suprabasal above the basal cell of the epidermis; Basal through the basal cells of the epidermis; Subepidermal beneath the basal keratinocytes, at the level of lamina lucida. Red asterisk indicates level of blistering.

by interference with intermediate filament turnover. In JEB, subepidermal blistering, at the level of the lamina lucida, is predominantly caused by mutations in integrin $\alpha 6\beta 4$ (*ITGA6*, or *ITGB4*), integrin $\alpha 3$ (*ITGA3*), type XVII collagen (*COL17A1*), or laminin 332 (*LAMA3*, *LAMB3*, *LAMC2*). Mutations in type VII collagen (*COL7A1*) underlie DEB, and blistering occurs below the lamina densa in the papillary dermis. Kindler Syndrome, due to mutations in kindlin-1 (*FERMT1*), is characterized by blistering at several levels. The level of blistering determines the subtype of EB. In general, blistering due to mutations in specific genes occurs at specific levels. Thus, the blister level can be used for diagnostics to guide the clinician to which protein is affected.

Incidence and prevalence of EB

The University Medical Center Groningen houses the national Center of Expertise for Blistering Diseases in the Netherlands and all Dutch patients with EB are seen in our clinic. Per July 2017, we had 590 EB patients in our database, of which 164 were patients with DEB. Similar to other countries, the prevalence of EB in the Netherlands is estimated to be ~1 in 22,000 births and the prevalence of the most severe form of DEB ~1 in 100,000 births.[16] Unfortunately, current treatment options for EB are merely symptomatic and prophylactic, and the average economic burden of EB has been estimated to be around €200,000 per year per patient.[16]

The mission of patient advocacy organizations, like the DEBRAs (https://www.debra.nl/; http://www.debra-international.org/), is to ensure that patients with EB have access to the best support and medical care, and to drive the development of new treatments and possible cures. One aspect of their work is to bring patients together to share experiences and to learn how to manage the burden of EB in a family. The severe forms of EB are devastating for both patients and parents. The patients suffer from severe pain and are in need of continuous care, which is mostly provided by the parents. The intensely painful process of changing the dressings has an impact on the lives of all involved that is difficult to describe. The German Pediatric Pain Centre filmed the family of the wonderful patient, Franz, and, in my opinion, succeeded in portraying the physical and psychological burden of a patient's life with EB. The video is entitled *Living with Epidermolysis Bullosa – Coping with Pain during Bandage Changes* and it is freely available (http://www.deutsches-kinderschmerzzentrum.de/en/about-us/videos/epidermolysis-bullosa-englisch/). There is also a film about Johnny Kennedy, *The boy whose skin fell off*. In his last few months, he decided to make a documentary about his life and death, together with film maker Patrick Collerton. The film is available on YouTube (https://www.youtube.com/watch?v=9wg8EtF5SJI).

Dystrophic epidermolysis bullosa and type VII collagen

DEB is due to mutations in the *COL7A1* gene, which encodes type VII collagen. To date,

more than 720 different mutations have been described that cause DEB, inherited in either a dominant or recessive fashion (DDEB and RDEB, respectively; http://www.hgmd.cf.ac.uk). The phenotype and severity of DEB varies widely, from only finger- and toenail involvement, to severe blistering of skin and mucosa accompanied by scar tissue formation. DEB is further classified based on the location of blister formation, and the involvement of skin and mucosae (Table 2). Recessive forms of DEB are generally more severe than dominant forms; in general, dominant DEB patients can have a normal life span. Patients affected by severe generalized DEB suffer severe mutilation of skin and mucosae and are in need of constant, life-long, care. An increased risk of developing aggressive squamous cell carcinomas is the main cause of death in young adult patients.[17-19]

The phenotypic outcome of DEB is strongly correlated to the functionality and quantity of type VII collagen present in the patient's skin.[20] The most severe subtype,

Table 2. List of clinical subtypes of dystrophic epidermolysis bullosa.

Inheritance	Clinical subtype	Abbreviation
Dominant	DDEB-generalized	DDEB-gen
	DDEB-acral	DDEB-ac
	DDEB-pretibial	DDEB-pt
	DDEB-pruriginosa	DDEB-pr
	DDEB-nails only	DDEB-na
	DDEB-bullous dermolysis of the newborn	DDEB-BDN
Recessive	RDEB-generalized severe	RDEB-gen sev
	RDEB-generalized intermediate	RDEB-gen intermed
	RDEB-inversa	RDEB-I
	RDEB-localized	RDEB-loc
	RDEB-pretibial	RDEB-pt
	RDEB-pruriginosa	RDEB-pr
	RDEB-centripetalis	RDEB-ce
	RDEB-bullous dermolysis of the newborn	RDEB-BDN

DDEB dominant dystrophic epidermolysis bullosa; RDEB recessive dystrophic epidermolysis bullosa.

RDEB-generalized severe, is caused by bi-allelic null mutations in *COL7A1,* which results in the complete absence of type VII collagen, whereas milder forms of RDEB are due to mutations that lead to expression of a partly functional type VII collagen.

The *COL7A1* gene (Table 3 and Appendix 1) encodes the 2,944 amino acid-long preproprotein pro-α1-type VII collagen (NP_000085.1), which consists of three major domains: an amino-terminal noncollagenous-1 domain (NC1), a triple helical domain (THD), and a carboxyl-terminal noncollagenous-2 domain (NC2). Three pro-α1-type VII collagens form a triple helix, which is typical for collagens.[21] Two triple helices then assemble in an

anti-parallel fashion and part of the carboxyl-terminus is cleaved to join the two triple helices into the mature type VII collagen, which is secreted. The mature type VII collagen then aggregates laterally into anchoring fibrils that connect the lamina densa to the papillary dermis.[22]

Table 3. Overview of the *COL7A1* gene (GRCh38.p7)

Chromosome position	3p21.31 (48,564,073 – 48,595,302)
Orientation	Antisense strand
Access # DNA	NG_007065.1
Access # mRNA	NM_000094.3
Access # Protein	NP_000085.1
Total length	31,088 bp
mRNA size	9,169 bp
cDNA size	8,835 bp
Amino acid sequence size	2,944 aa
Average exon size	80 bp
Average exon size NC1	145 bp
Average exon size THD	54 bp
Average exon size NC2	135 bp
Largest exon size*	201 bp (exon 73)
Shortest exon size	27 bp (exons 29, 37, and 56)
Average intron size	187 bp
Average intron size NC1	198 bp
Average intron size THD	177 bp
Average intron size NC2	271 bp
Largest intron size	1,293 bp (intron 64)
Shortest intron size	70 bp (intron 30)
Phase of reading frame NC1	2**
Phase of reading frame THD	1
Phase of reading frame NC2	2
# of skipable exons	107 (~92%)***
# of exons encoding Gly-X-Y only	60 (337 Gly-X-Y repeats, collectively)
Total Gly-X-Y repeats	454

*Exon 118 comprises 350 bp, however, it encodes only 14 amino acids.
**Except exons 3, 4, 7 and 25 that start at phases 3, 1, 1, and 1, respectively.
***Not counting exon 1 or exon 118 in calculating the percentage.
NC1 noncollagenous-1 domain; THD triple helix domain; NC2 noncollagenous-2 domain; bp base pairs; aa amino acids

In order to form the triple helix, the THD encodes a highly repetitive amino acid sequence. The amino acid glycine is essential for forming the THD, which, for the most

part, consists of a Glycine-Xaa-Yaa sequence repeat, where the glycine is mandatory and the 'X amino acid' and 'Y amino acid' are often proline and hydroxyproline.[23] This highly structured glycine-repeat is interrupted 19 times and the largest interruption is located in the middle of the THD.[24] This region (39 amino acids long), encoded by exons 71 and 72, is called the 'hinge domain' due to its so-called intrinsically disordered structure, which provides flexibility to the otherwise rigid triple helical structure.[25] A single glycine-substitution in the THD can impair the ability to form a stable triple helix and is the main cause of dominant DEB.[26]

Splicing and translation

The complex process of splicing by the spliceosome relies on consensus sequences that define exons and introns, and on small nuclear ribonucleoproteins (snRNPs) that bind to these sequences, to form the spliceosome (Figure 3).[27] To date, two spliceosomes have been identified, the major and minor spliceosomes. The major spliceosome accommodates splicing of introns that start with the 5'-end splice donor sequence GU, and 3'-end splice acceptor sequence AG, while the minor spliceosome splices introns with a donor sequence AU and an acceptor sequence AC.[28] The major spliceosome consists of snRNP U1, U2, U4, U5, and U6. In short, the U1 binds to the 5'-GU sequence of the intron and associates with U2, which binds to the so-called branch point A in the intron. The proximity of U1 to U2 attracts the snRNPs U4, U5, and U6, which subsequently assemble with U1 and U2 to form the spliceosome. The snRNPs U1 and U4 then disassemble and are reused. The active spliceosome comprises snRNPs U2, U5, and U6, and catalyzes the splicing. First, the branch point is joined with the 5'-GU to form a lariat. Subsequently, the exons are joined and the lariat intron is released, yielding the mature mRNA.

The minor spliceosome is highly similar to the major spliceosome, except that it comprises the snRPS U11, U12, U4atac, and U6atac, which are functional analogues to U1, U2, U4, and U6, respectively.[28]

The 31,088 bp long gene, *COL7A1* DNA (NG_007065.1), gets transcribed into precursor-messenger RNA (pre-mRNA), comprising 117 non-coding introns and 118 coding exons. Splicing results in a 9,169 bp long mRNA (NM_000094.3), which is subsequently translated into the 2,944 amino acid sequence, i.e. the pro-alpha type VII collagen protein that is released into the rough endoplasmic reticulum where post-translational modifications take place.[22]

Physiological and induced alternate splicing

Although the mechanism of splicing by the spliceosome in the nucleus is well understood,[29] the recruitment of the spliceosome and the mechanisms of alternate splicing are not fully known. In general, non-spliceosomal RNA-binding proteins bind to the pre-mRNA and can thereby enhance or silence the recruitment of spliceosomal snRNPs. Exonic

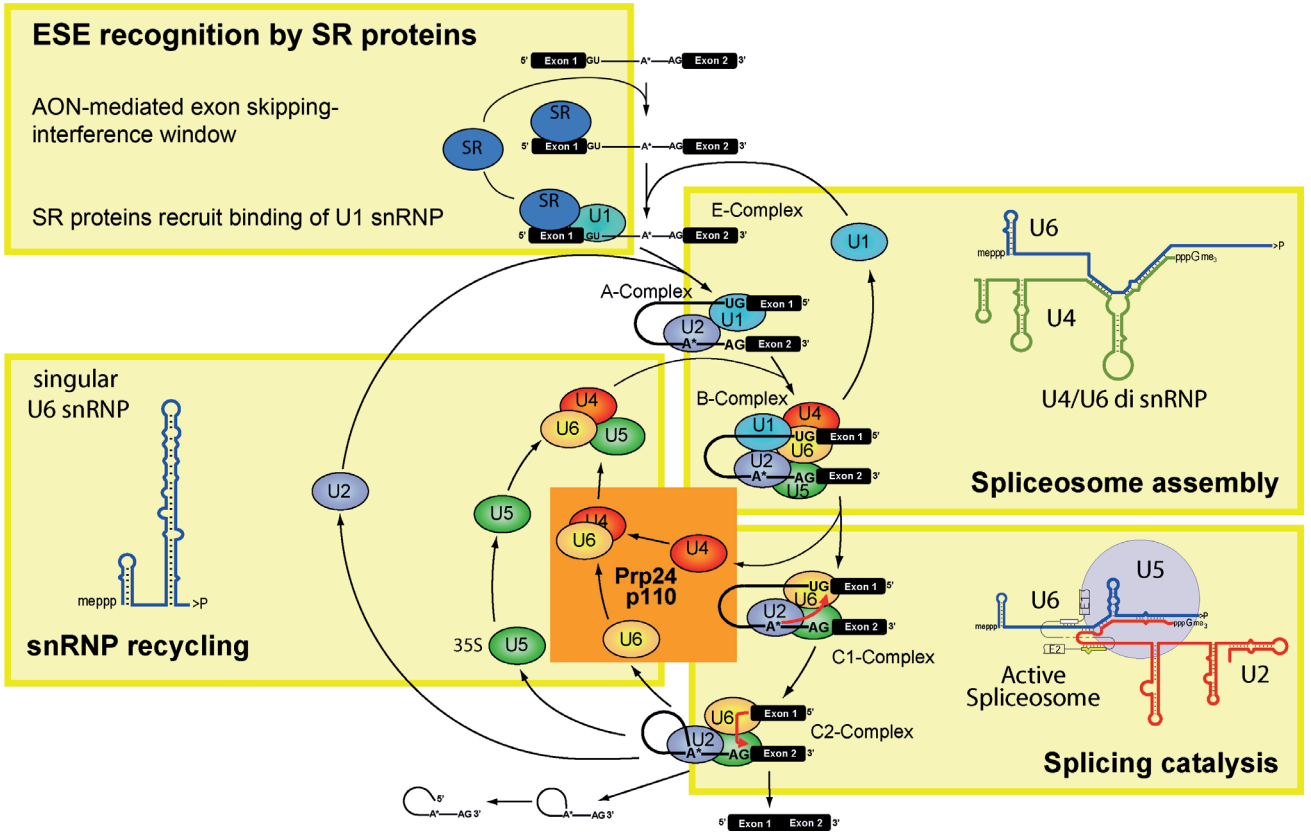

Figure 3. Schematic overview of the splicing process. Four major steps involved in the splicing process are depicted in yellow frames. First, SR-proteins (dark blue) bind to the pre-mRNA and recruit snRNPs U1 (light blue) that binds to the splice acceptor site, and U2 (purple) that binds to the A-polypyrimidine (A*) tract upstream of the acceptor site to form the E-Complex. U1 and U2 together create the A-Complex. Subsequently, U4 (red) assembles U6 (yellow) and U5 (green) to form the B-Complex, and U2 creates a lariat by cleaving the splice donor site and connecting it to A* in the C1-Complex. Finally, the C2-Complex cuts the splice acceptor site and joins the exons together. The lariat is released and snRNPs are recycled throughout the process (indicated by arrows). Prp24 and p110 assemble the U4-U6 complex.
AON; antisense oligonucleotide, SR; serine-arginine rich protein, snRNP; small nuclear ribonuclear proteins, U1-6; snRNP U1-6.

splice enhancer (ESE) or -silencer (ESS) sequences, and intronic splice enhancer (ISE) or -silencer (ISS) sequences, are motives for RNA-binding proteins that influence splicing.[30] As we now know, alternate splicing seems to be the main source of genetic diversity and deep sequencing studies recently showed that there are on average 3.4 isoforms per gene.[31] It is estimated that between 95%[32, 33] and 100%[34, 35] of protein-coding genes encode multiple isoforms.

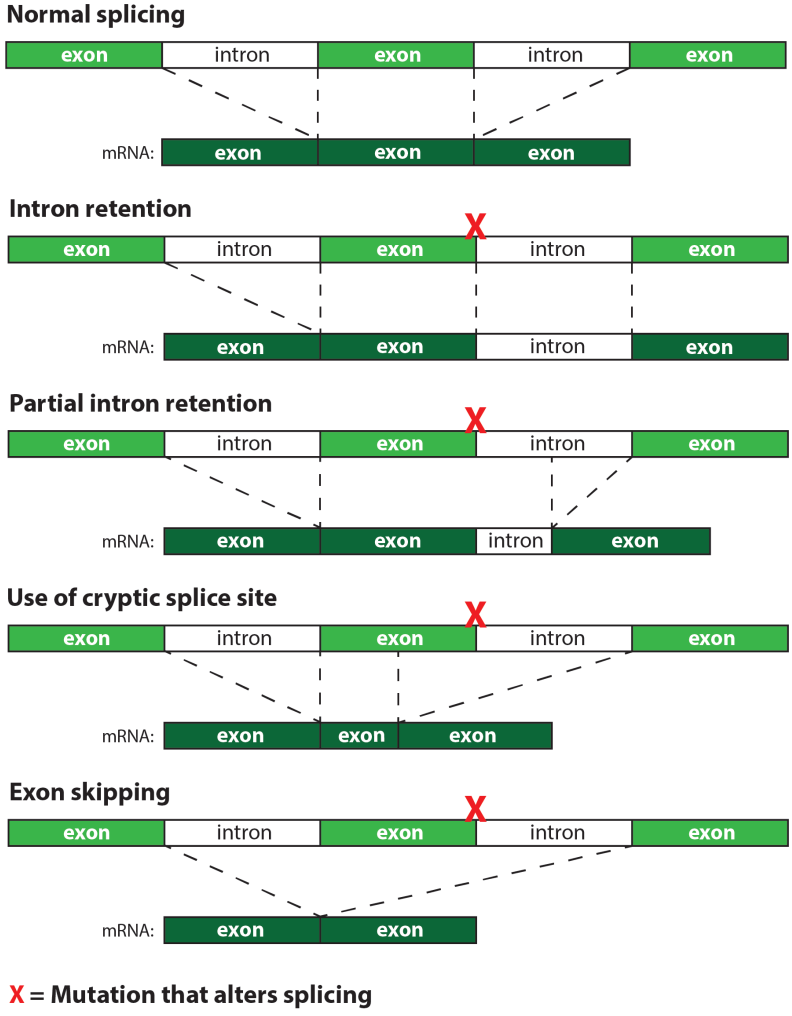

Figure 4. Mutations that affect splicing can result in different outcomes. From top to bottom: **Normal splicing** splicing as expected. **Intron retention** the complete intron is retained in the mRNA. **Partial intron retention** an intronic cryptic splice site is used resulting in partial intron retention. **Use of cryptic splice site** an exonic cryptic splice site is used resulting in a truncated exon. **Exon skipping** the entire exon including surrounding introns is spliced out of the mRNA.

Alternate splicing can also be induced by mutations that are located in splice sites or splice signal sequences, with an estimated 10-15% of all diseases causing point mutations that influence splicing.[36, 37] Depending on the locus and surrounding sequence, multiple outcomes can be conceived (Figure 4). Alternate splicing can result in intron retention, partial intron retention, the use of an exonic cryptic splice site or the skipping of an entire exon.

Antisense oligonucleotide-mediated exon skipping

The naturally occurring skipping of an entire in-frame exon has been the inspiration to use exon skipping as a therapeutic approach for Duchenne muscular dystrophy (DMD) since the late 1990s.[38] DMD is caused by out-of-frame exon deletions or null mutations in the *DMD* gene that lead to the complete absence of dystrophin.[39] It is characterized by progressive deterioration of muscle tissue, which inevitably results in the loss of motor skills and eventually in death.[40] The less severe Becker muscle dystrophy is caused by in-frame deletions that lead to expression of a slightly shorter, but at least partly functional, dystrophin.[41] The in-frame deletions observed in Becker muscular dystrophy were the basis for the hypothesis that using exon skipping to restore the reading frame in patients with DMD could ameliorate the phenotype towards the much milder Becker muscular dystrophy.[42] Successful pre-clinical and clinical exon skipping in the *DMD* gene, and the structural similarity of the protein dystrophin and type VII collagen, led to the idea that exon skipping might also be beneficial for DEB. The highly repetitive THD linker region seemed a perfect candidate for using exon skipping as a therapeutic approach.

Antisense oligonucleotide (AON)-mediated exon skipping relies on Watson-Crick base pairing and steric hindrance of RNA-binding proteins by AONs.[43] AONs are specifically designed to bind to splice signal sequences of the pre-mRNA. Interestingly, exonic splice enhancer sequences, especially so-called rescue-ESEs, were identified as the primary target of AONs to induce exon skipping rather than consensus splice sites.[44] When specific RNA-binding proteins are inhibited from binding to the pre-mRNA, the exon is no longer defined as an exon and it is spliced out together with its surrounding introns (Figure 5). In order to do this, the AONs must not provoke RNase-H-activity and should be able to bind with adequate affinity to the target RNA.[45] To that end, various chemical modifications have been developed over the last decades, which affect the ribose sugar and/or backbone of the nucleic acid sequence and have great influence on the binding affinity.[46] The chemistry is an important characteristic of the AON and has a major impact on pharmacokinetics and pharmacodynamics.[47-50] Therefore, for each target tissue, the most suitable chemistry should be investigated. AONs delivered systemically, accumulate rapidly in the liver and are cleared through the kidneys. In this thesis, we used 2'-*O*-methyl phosphorothioate (2OMePS) modified RNA oligonucleotides, but there are several chemical modifications available for AON therapeutics. The two most promising

alternative chemistries for the 2OMePS are the phosphorodiamidate morpholino (PMO) and 2-*O*-Methoxyethoxyethyl (MOE) (Figure 6). The 2OMePS contain a modified ribose sugar at the 2'- carbon atom that are linked to a methyl group via an oxygen atom. The linkages, or backbone, are modified with a sulfur atom replacing an oxygen atom, transforming the phosphodiester into a phosphorothioate. Phosphorothioate linkages were discovered in the late 1960s to early 1970s by Prof. Eckstein and colleagues; they make the oligonucleotides resistant to RNase-H degradation, which, of course, is essential for splice-modulating antisense technology.[51]

Since 2007, numerous clinical trials with splice switching antisense oligonucleotide therapeutics have been performed. At the start of my research work in 2013, there were four oligonucleotide therapeutic drugs approved by the American Food and Drug Association (FDA) and/or European Medicines Agency (EMA). As per July 2017, this number has increased to six oligo drugs (Table 3), two of which, are splice-switching oligonucleotides. The first splice-switching oligonucleotide, Eteplirsen (Sarepta Therapeutics), is an exon-skipping AON to treat patients with Duchenne muscular dystrophy. It induces skipping of exon 51, and thereby restores the reading frame in patients that carry out-of-frame deletions of exons preceding exon 51, e.g. an exon 50 deletion.[52] The second splice-switching oligo drug, Nusinersen (Ionis Pharmaceuticals) is an AON designed to treat spinal muscular atrophy and it was recently approved by both the FDA and EMA. Nusinersen switches the splicing of the *SMN2* gene to include its exon 7, which is normally excluded from the mRNA, and it thereby mimics the *SMN1* gene.[53] Nusinersen basically aims to include an exon and therefore does the opposite of exon skipping. In the general discussion at the end of this thesis, I will show what we can learn from these approved RNA therapeutics and, just as important, what we can learn from the RNA therapeutics that did not receive market authorization, such as Drisapersen (Biomarin), another AON aimed at skipping exon 51 of the *DMD* gene.

Table 4. Overview of FDA and/or EMA decisions on submitted oligonucleotide therapeutics for marketing authorization

Name	Developing company	Disease	Mode of action	Chemistry	Decision
Fomivirsen (Vitravene)	Ionis Pharmaceuticals	Cytomegalovirus retinitis	Inhibition of translation	DNA phosphorothioate	Approved FDA: 1998 Approved EMA: 1999
Pegaptanib (Macugen)	NeXstar	Neovascular age-related macular degeneration	Antagonistic binding to target protein	2′-O-methyl, 2′-fluorinated phosphorothioate*	Approved FDA: 2004 Approved EMA: 2006
Mipomersen (Kynamro)	Ionis Pharmaceuticals	Familial hypercholesterolemia	RNase H induced RNA degradation	2′-O-methoxyethoxy phosphorothioate gapmer	Approved FDA: 2013 Declined EMA: 2013
Defibrotide (Defitelio)	Jazz Pharmaceuticals	Severe hepatic veno-occlusive disease in haematopoietic stem-cell transplantation therapy	Non-specific protein interactions / unknown	Polydisperse mixture of phosphodiester nucleotides	Approved FDA: 2016 Approved EMA: 2013
Drisapersen (Kyndrisa)	BioMarin	Duchenne muscular dystrophy	Splice switching (exon skipping)	2′-O-methyl phosphorothioate	Declined FDA 2016 Withdrawn EMA: 2016
Eteplirsen (EXONDYS 51)	Sarepta Therapeutics	Duchenne muscular dystrophy	Splice switching (exon skipping)	Phosphorodiamidate morpholino	Approved FDA:2016 Under evaluation EMA
Nusinersen (Spinraza)	Ionis Pharmaceuticals	Spinal muscular atrophy	Splice switching (exon inclusion)	2′-O-methoxyethoxy phosphorothioate	Approved FDA: 2016 Approved EMA: 2017

* 3′-3′ deoxythymidine and 5′-linked with a 40 kDa polyethylene glycol substituent

OUTLINE AND AIM OF THIS THESIS

The aim of my thesis is to investigate the feasibility and impact of exon skipping as therapy for EB. The thesis is divided into two parts: in the first part, AON-mediated exon skipping as a therapeutic approach for DEB is studied; in the second part, exon skipping therapy for EB is placed in perspective by investigating the natural exon skipping that causes DEB and by describing case studies that illustrate the feasibility of exon skipping for other types of EB.

In **Part one**, in **Chapter 2**, we describe investigations into the feasibility of AON-mediated exon skipping as systemic treatment for DEB. In **Chapter 3**, we analyzed the functional consequences of exon skipping on type VII collagen in *in vitro* and *in vivo* settings. In **Chapter 4**, we describe how we investigated the origin of type VII collagen and anchoring fibrils, i.e. mouse or human, in two widely used mouse models with human skin grafts. In **Chapter 5**, we review the advantages and disadvantages of different RNA-based therapeutic strategies for genodermatoses, including AON-mediated exon skipping.
In **Part two,** we explore naturally occurring exon skipping in DEB, JEB, and EBS and studied the genotype-phenotype correlation of exon skipping. **Chapter 6** provides an extensive review of the literature on natural exon skipping in DEB and adds a case series study. In examining the genotype-phenotype correlation of exon skipping, we aimed to provide insight into the expected clinical benefit of exon skipping for patients with DEB. In **Chapter 7**, we investigated a case where exon skipping in the *COL17A1* gene ameliorated the expected phenotype in JEB, showing clinical benefit from exon skipping for another EB type. In **Chapter 8**, we present a case in which exon skipping in the *KRT5* gene led to EBS, highlighting how exon skipping can have not only beneficial effects. And in **Chapter 9**, we report on a new isoform of plectin, which is differentiated from other *PLEC* isoforms by alternate splicing of exon 8. This emphasizes the need to fully characterize the *COL7A1* gene in order to better assess the on- and off-target effects of AONs.

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Part one

Exon skipping as therapeutic approach